

Figure 1: Cloning by *in vitro* assembly and transformation of competent *Bacillus*

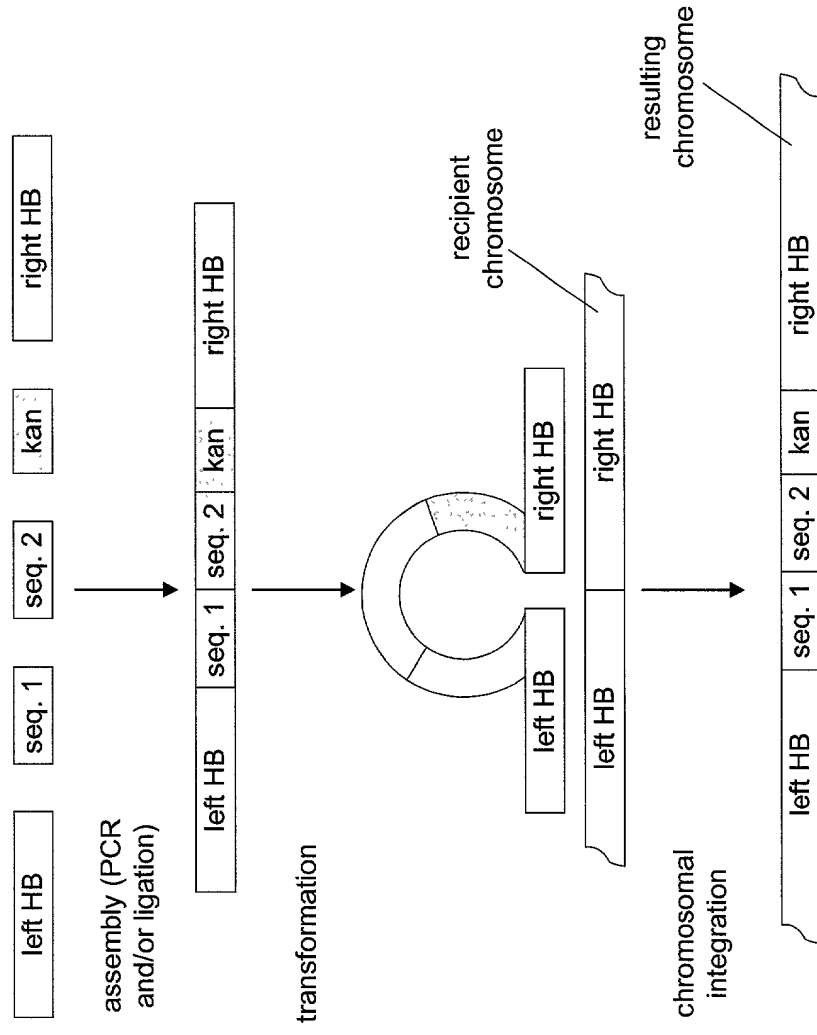


Figure 2: Adding non-homologous flanks increases the transformation efficiency.

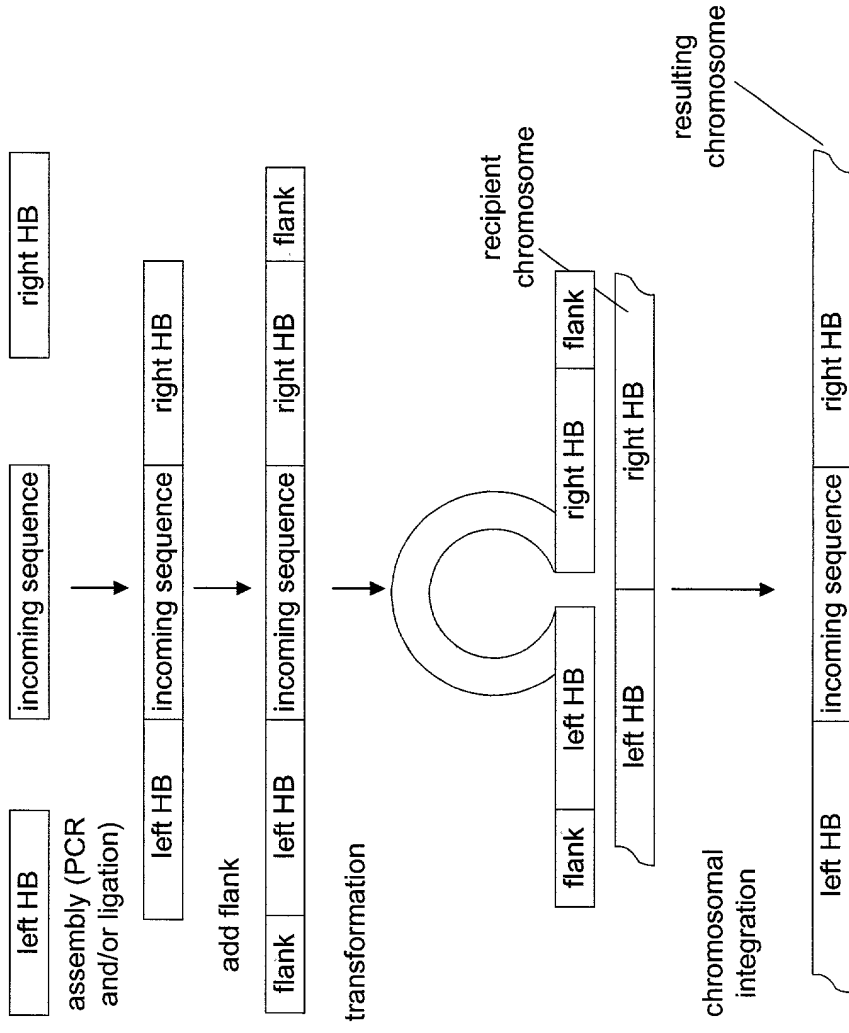


Figure 3: PCR mutagenesis of a region of the Bacillus chromosome

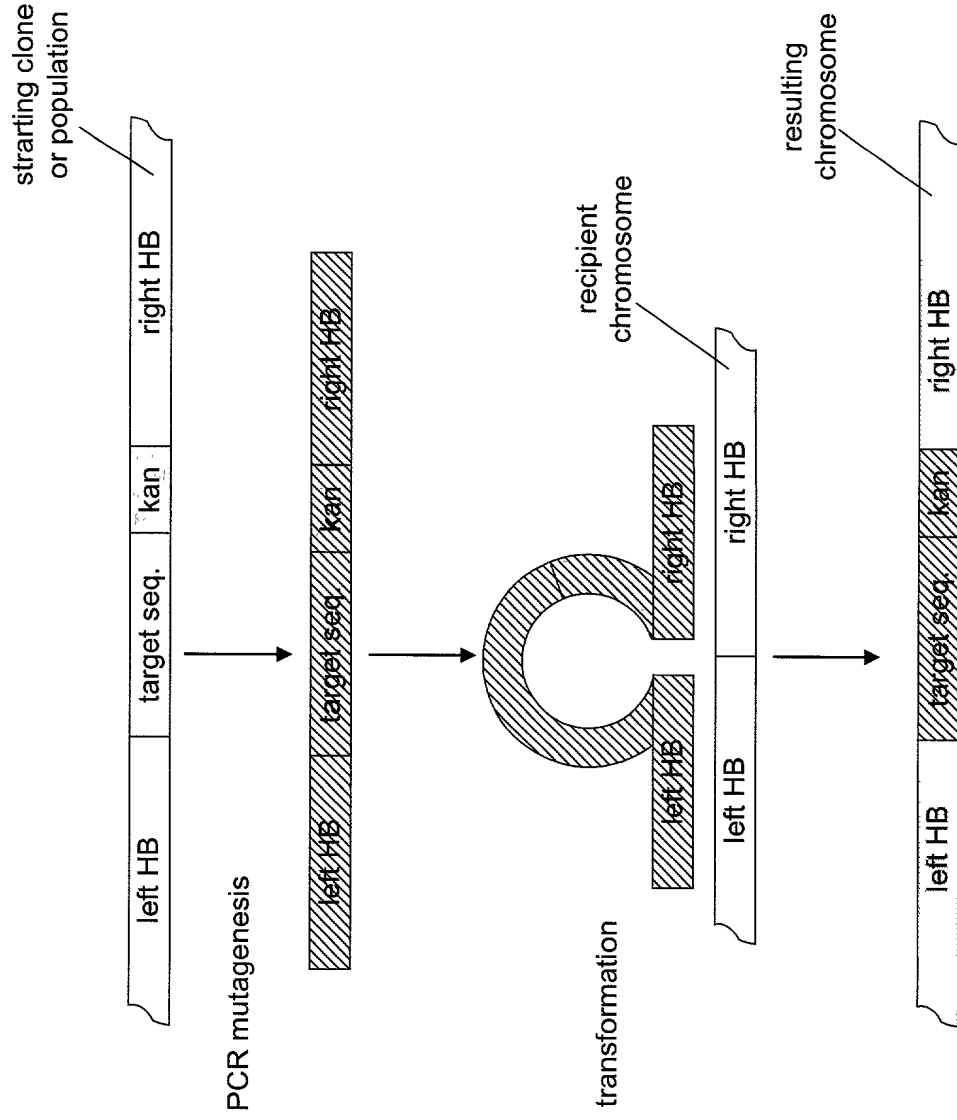


Figure 4: Maximizing the homology between the transforming DNA and the target region of the chromosome increases the transformation efficiency.

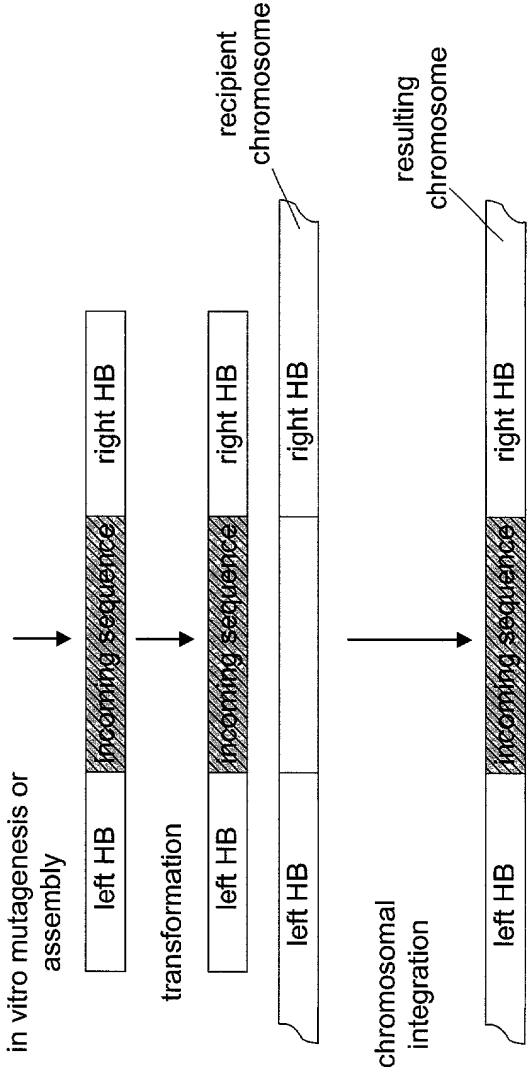


Figure 5: Using a competent host that carries an inactive version of the marker gene, used to select transformants

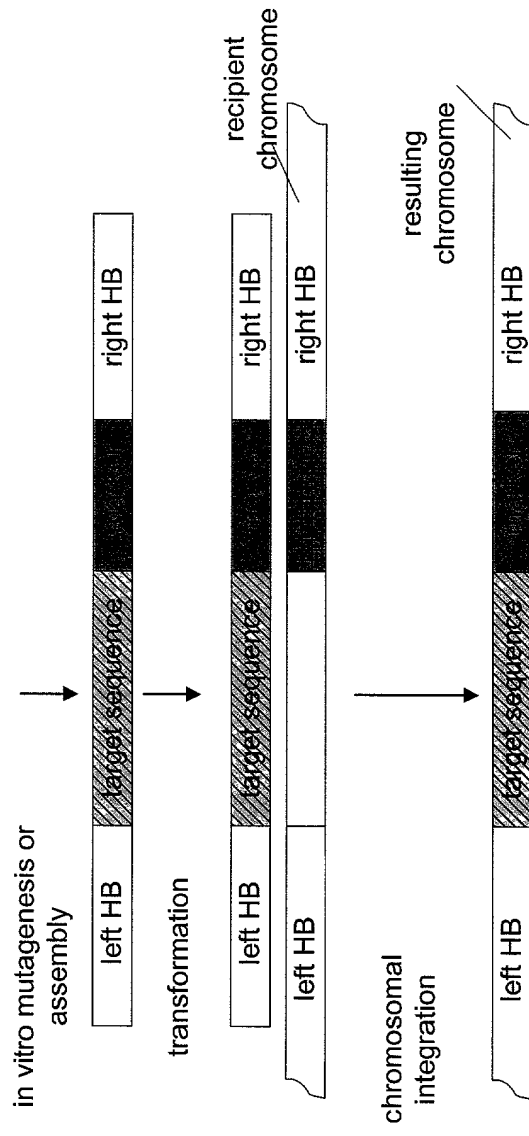


Figure 6: General structure of transforming DNA

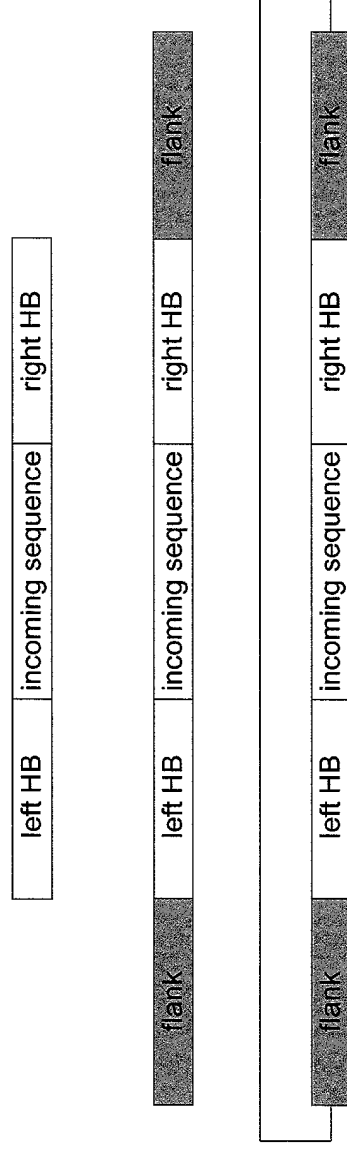
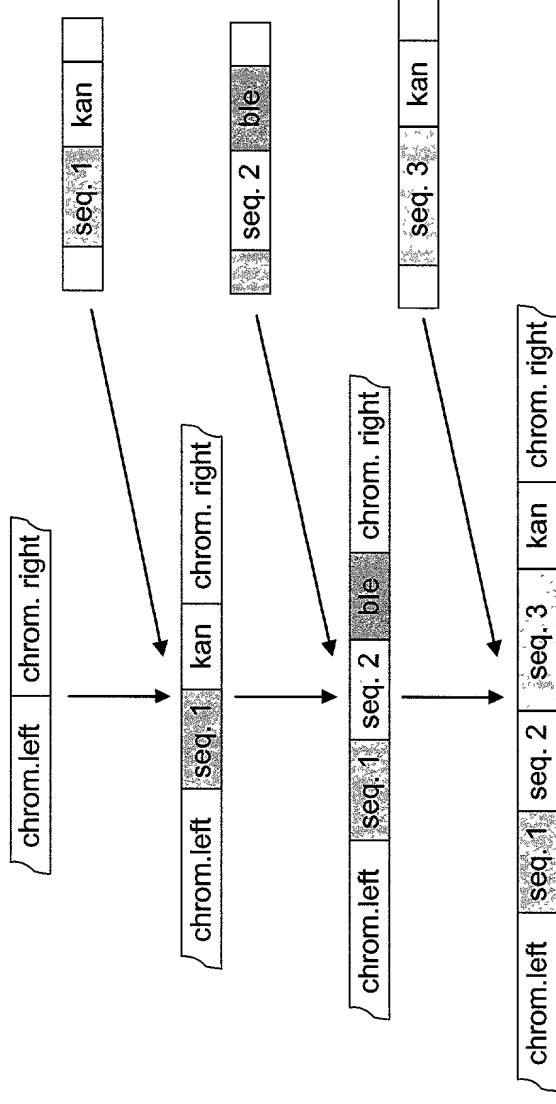


Figure 7: *Bacillus* strain construction by iterative marker replacement



Advantages

- Replacement allows one to verify that construct went into target locus.
- Large sequences can be assembled in vivo.
- Avoids repeat PCR amplification
- Entire construct can be moved by transduction or transformation.
- Two markers are sufficient to introduce an unlimited number of genes.

Figure 8A

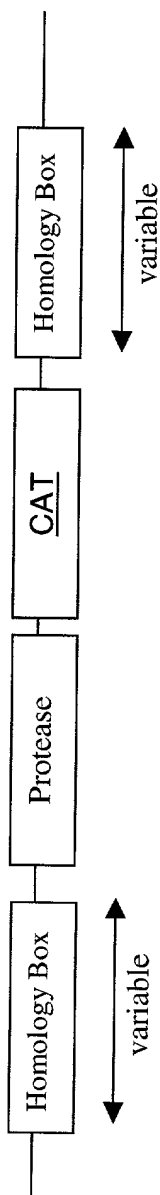
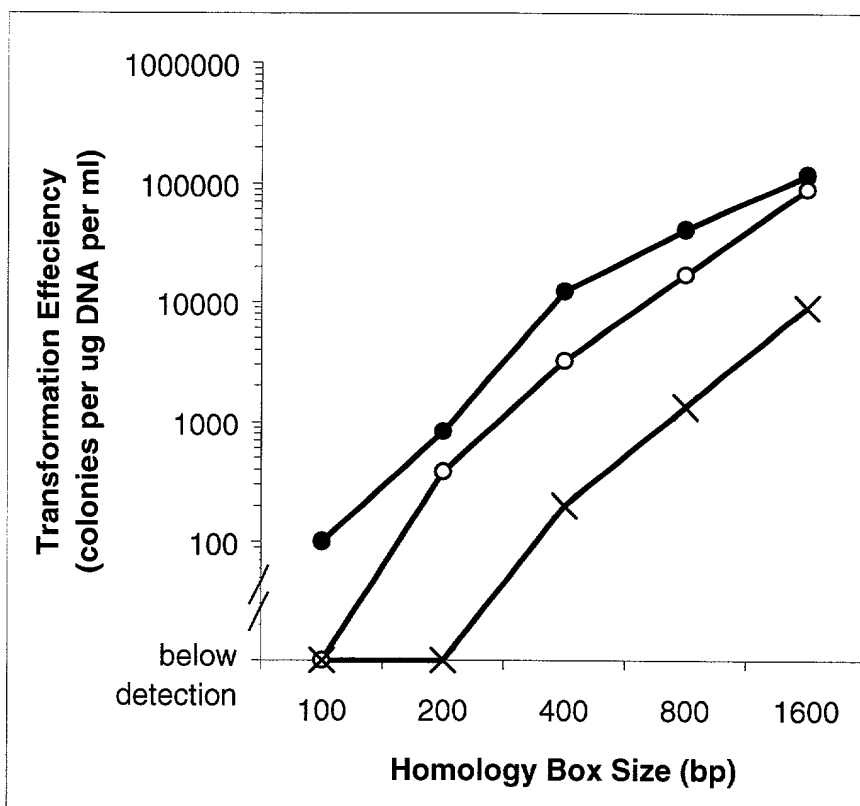


Figure 8B. Optimization of homology box size



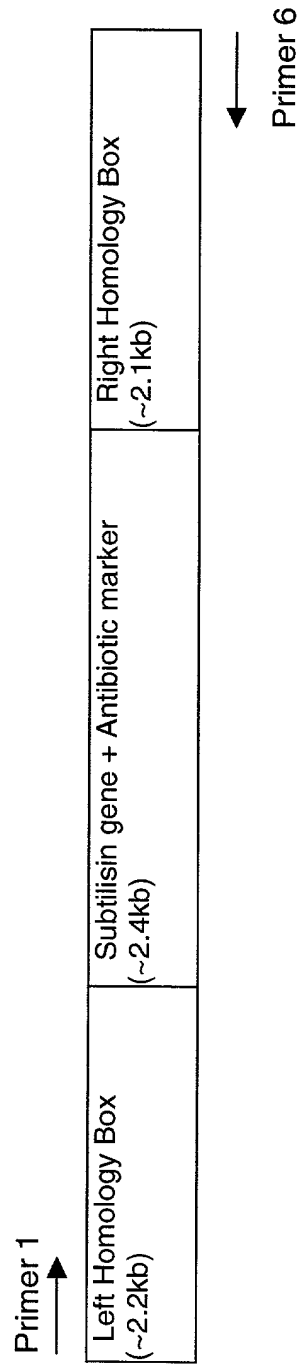


Figure 9

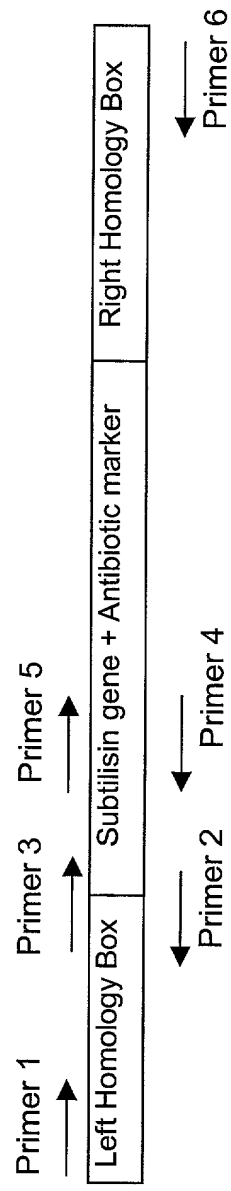


Figure 10

Figure 11

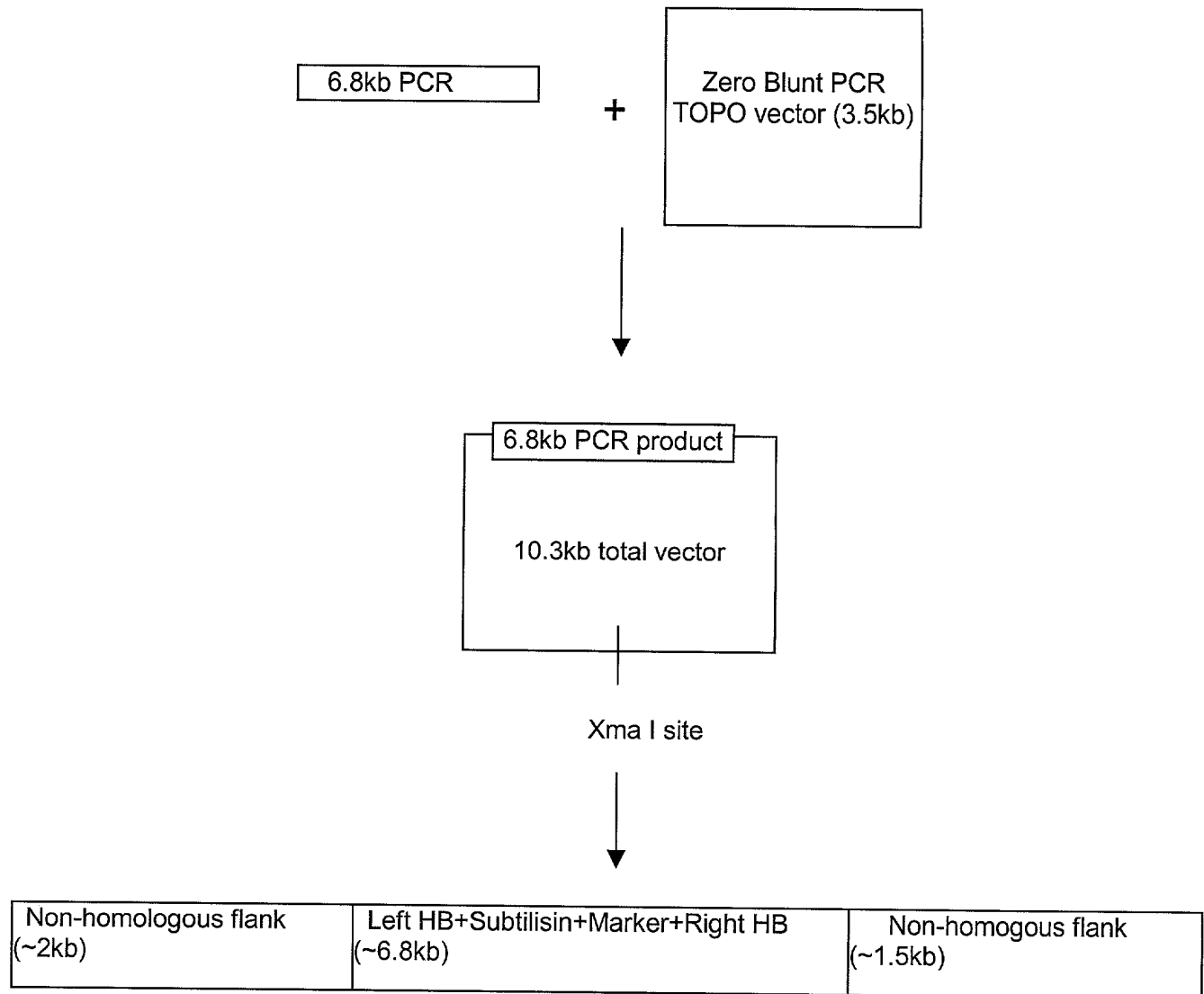


Figure 12

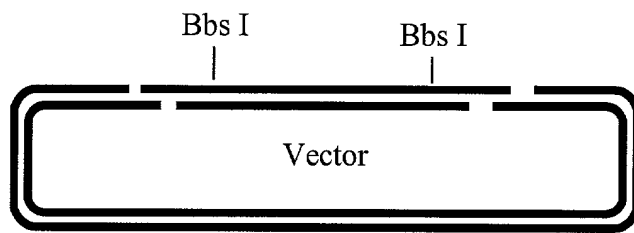
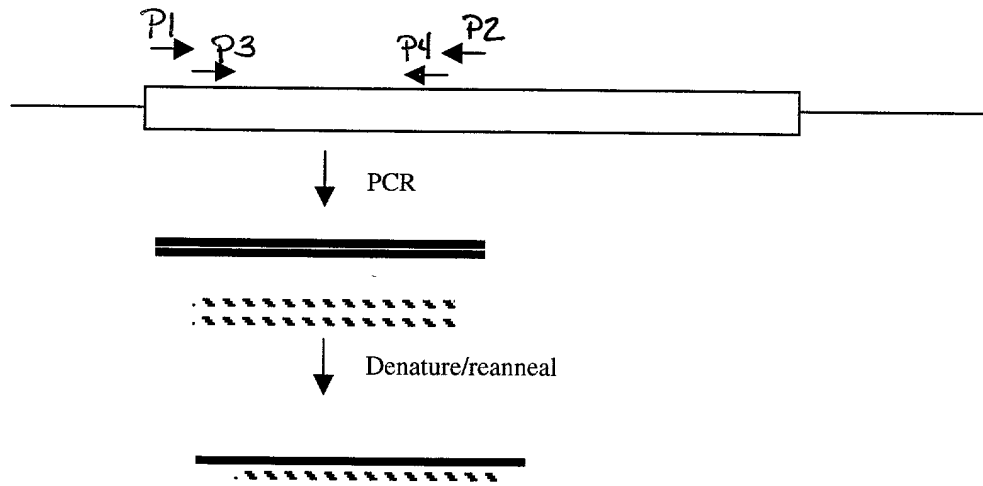
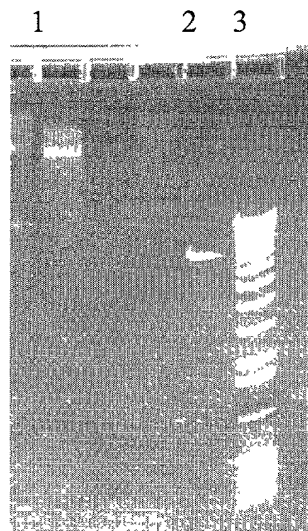


Figure 13



0992461.091001
T00T00" T0T000

Figure 14



Parameter	Value	Unit
Temperature	25.0	°C
Pressure	1.0	atm
Flow rate	1.0	L/min
Concentration	0.1	mol/L
pH	7.0	
Wavelength	254	nm
Scan rate	1.0	nm/min
Integration time	1.0	s
Resolution	0.5	nm
Slit width	1.0	mm
Detector	Photodiode array	
Software	Chromatography	
Hardware	PC	
Manufacturer	Agilent	
Model	1100	
Version	1.0	
Year	2000	
Author	J. H. Kim	
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Subject	Chromatography	
Keywords	Chromatography, HPLC, GC, MS	
Abstract	Chromatography is a technique used to separate mixtures into their individual components. It is widely used in chemistry, biology, and medicine. The most common types of chromatography are gas chromatography (GC) and liquid chromatography (LC). GC is used to separate volatile compounds, while LC is used to separate non-volatile compounds. Both GC and LC can be coupled with mass spectrometry (MS) to identify the components of a mixture. Chromatography is a powerful tool for analyzing complex mixtures and is essential for many scientific and industrial applications.	

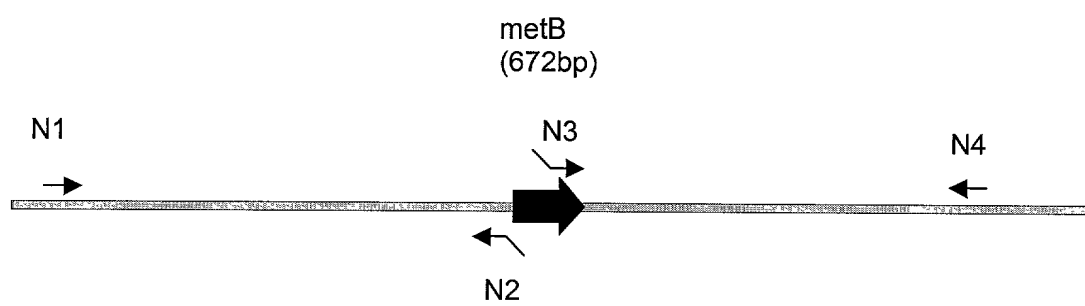


Figure 15